Trichodysplasia Spinulosa – A Newly Described Folliculocentric Viral Infection in an Immunocompromised Host

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This is a case report of an immunocompromised individual who presented with progressive alopecia, friable follicular spinous processes, and erythematous, indurated papules. Examination of skin biopsies using light microscopy and immunohistochemistry revealed pathologic changes of the follicular inner root sheath epithelium with dystrophic trichohyaline granules. Electron microscopy of thin sections of tissue revealed intracellular viral particles with a size and appearance consistent with those in the Papovaviridae family. Electron microscopy of negatively stained extract from a homogenized lesion also demonstrated icosahedral viruses with papovavirus morphology. We believe this is a previously unreported folliculocentric viral infection in an immunosuppressed human host and have termed this entity ''trichodysplasia spinulosa''. ***Key words: hair follicle/viral infection/immunosuppression. Journal of Investigative Dermatology Symposium Proceedings 4:268–271, 1999***

This is a case report of a folliculocentric viral infection occuring in an immunocompromised individual who presented with progressive alopecia and erythematous, indurated papules. We believe this is a previously unreported condition in a human host and have termed this entity ''trichodysplasia spinulosa''.

CASE REPORT

The patient is a 44-y-old Caucasian male who underwent a combined renal/pancreas transplant for treatment of type I diabetes mellitus 3 y prior to presentation. He was placed on a standard immunosuppressive regimen consisting primarily of FK-506 (tacrolimus), azathioprine, and prednisone. Seven months prior to presentation, he developed alopecia of both eyebrows acutely. This was followed by the development of eruptive papules predominantly located on the nose, ears, and forehead. The alopecia progressed, and within the next several months he lost most of his body hair with only sparse terminal scalp and pubic hair remaining. Erythematous, indurated papules coalesced into plaques over the face and ears. The patient's overall appearance was dramatically changed from 1 y earlier, with a development of ''leonine'' facies (**Fig 1*a***). There was eyebrow, eyelash, and scalp alopecia. Small, friable, white spines projected from the follicular orifices in the alopecic areas (**Fig 1*b***).

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Abbreviations: HPV, human papillomavirus; PV, papillomavirus; TBS, tris buffered saline.

MATERIALS AND METHODS

Biopsies Light microscopy was performed on vertically and horizontally sectioned 5 mm cylindrical punch biopsies from several clinical sites. Tissue was also sent for fungal, bacterial, and mycobacterial cultures.

Immunohistochemistry Cryosections of skin biopsies were fixed in acetone at –20°C, rinsed in tris buffered saline (TBS) and blocked for nonspecific binding with 2% normal goat serum in TBS. Indirect immunofluorescence was performed applying primary antibodies: AE-15 undiluted, for localization of trichohyalin; antikeratin 14 diluted 1:50 to label basal keratinocytes; and proliferating cell marker Ki67 (DAKO, Carpenteria, CA) at 1:200 dilution for 1 h. Sections were subsequently labeled with fluorescently tagged secondary antibodies, donkey antimouse texas red (1:500) (Jackson, West Grove, PA), and goat antirabbit FITC (1:300) (Vector, Burlinghame, CA). Tissue sections were counterstained with 4′, 6-diamidino-2-phenylindole (DAPI) allowing visualization of nuclei by the emission of blue fluorescence. Specimens were visualized on a Nikon Microphot-SA microscope equipped with epi-fluorescence illumination. Red, green, and blue fluorescence images were sequentially captured on a monochrome Photometrics SenSys CCD camera using IPLab Spectrum (Scanalytics, Vienna, VA) image acquisition software. Serial paraffin embedded skin sections were rehydrated, and immunohistochemistry performed using a broadly cross-reactive rabbit polyclonal antibody directed against highly conserved papillomavirus group-specific antigens (DAKO, CA) and a panel of mouse monoclonal antibodies directed at specific conserved viral epitopes (AU1-AU6 and 1H8) as previously described (Lim *et al*, 1990; Jenson *et al*, 1997). A bovine cutaneous fibropapilloma containing bovine papillomavirus type II was used as a positive control.

Immunoperoxidase staining for BK polyomavirus was performed using a monoclonal primary antibody directed against the BK virus large-T antigen (Chemicon, Temecula, CA), which reportedly does not cross-react with other papovaviruses, including JC virus (Marshall *et al*, 1991). The avidin-biotinylated peroxidase staining technique was employed, with diaminobenzidine as chromogen.

Electron microscopy Frozen tissue was quick thawed and fixed in half-strength Karnovsky's fixative for 24 h, postfixed in osmium tetroxide, en

Figure 1. Clinical presentation. (*a*) Patient with "leonine" facies. Scalp, eyebrow, and eyelash alopecia is present. (*b*) Patient's nose showing small, friable, white spines projecting from the follicular orifices.

bloc stained in uranyl acetate, dehydrated through graded ethanol and propylene oxide, and embedded in epoxy resin. Thin sections were collected onto copper grids, stained sequentially with uranyl acetate and lead citrate, and observed using a Philips 420 STEM operated at 60 kV in the transmission mode. A 3 mm^3 piece of tissue from one of the biopsies was ground in a morter with pestle. Water was added and the cell debris pelleted by centrifuging at $1000 \times g$. The supernatant was ultracentrifuged at $100\,000 \times g$ for 50 min. The ultracentrifuge pellet was resuspended in 20 μl water and placed onto a Formvar and carbon-coated grid. It was negatively stained in 2% uranyl acetate and viewed in a Philips EM300 electron microscope.

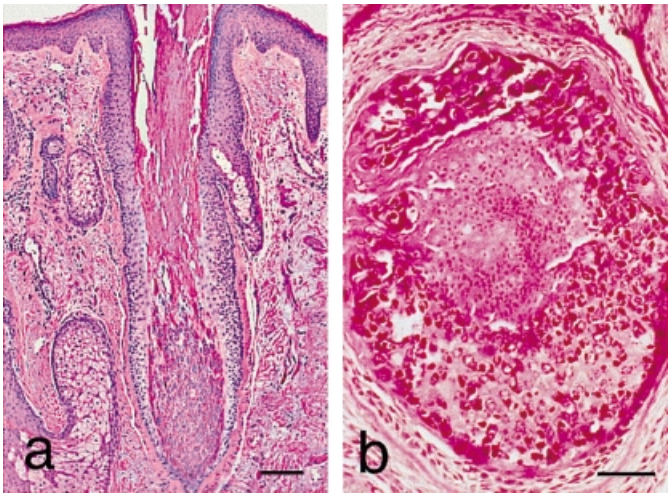

Figure 2. Light microscopy. (*a*) Vertical section from nose biopsy. The follicles are dramatically expanded and there is dilatation and keratotic plugging of the infundibula (hematoxylin and eosin; *scale bar*: 100 μm). (*b*) Horizontal section from nose biopsy. There is marked dystrophy of the inner root sheath with enlarged, irregular, trichohyaline granules (*arrow*) (hematoxylin and eosin; *scale bar*: 50 μm).

Detection for human papilloma virus (HPV) subtypes using polymerase chain reaction (PCR) DNA was extracted from the biopsy specimens and analyzed for HPV DNA by a consensus primer PCR amplification and dot blot hybridization technique using mixtures of oligonucleotide probes complementary to 18 viral subtypes in the following combinations: 6/11, 16, 18, 31/33/35/39, 40/42/53/54, 51/52/55/58, and 45/56 as previously described (Kuypers *et al*, 1993). The biotin-labeled probes were detected on X-ray film using chemiluminescent substrates.

RESULTS

Biopsies Light microscopy revealed pathologic changes limited to the follicular epithelium. The follicles were dramatically expanded, and there was dilatation and keratotic plugging of the infundibula (**Fig 2*a***). There were no normal hair shafts present. The inner root sheath epithelium demonstrated marked dystrophy with enlarged, irregular, trichohyaline granules, acantholysis, and numerous apoptotic cells (**Fig 2*b***). Fungal, bacterial, and mycobacterial cultures and special stains from the tissue were repeatedly negative.

Immunohistochemistry Using the antibody AE-15, a marker for trichohyaline granules (O'Guin *et al*, 1992), revealed a dramatic expansion of expression in the inner root sheath (**Fig 3*b***) compared with the expression pattern observed in follicles from a normal control (**Fig 3*a***). Ki 67 expression using an antibody directed against proliferating cell nuclear antigen, revealed a dramatic increase in protein levels (**Fig 3*d***) compared with a normal control (**Fig 3*c***), indicating marked cell proliferation activity in the affected follicles. An antibody for highly conserved papillomavirus (PV) epitopes that broadly cross-reacts phylogenetically with mammalian and avian PV and a panel of monoclonal antibodies directed at specific PV epitopes, failed to detect the presence of PV antigens in any of the tissue sections tested. An antibody for the large-T antigen of BK polyomavirus failed to detect the presence of BK virus in any of the tissue sections tested.

Electron microscopy Transmission electron microscopy of thin sections of skin revealed small (38 nm), regular, intracellular virus particles (**Fig 4*a***). The negatively stained preparation of ground tissue contained 38 nm particles with the bumpy appearance of viruses in the Papovaviridae family (**Fig 4*b***).

Detection for HPV subtypes using PCR PCR analysis of tissue with 18 oligonucleotide probes complementary to HPV viral

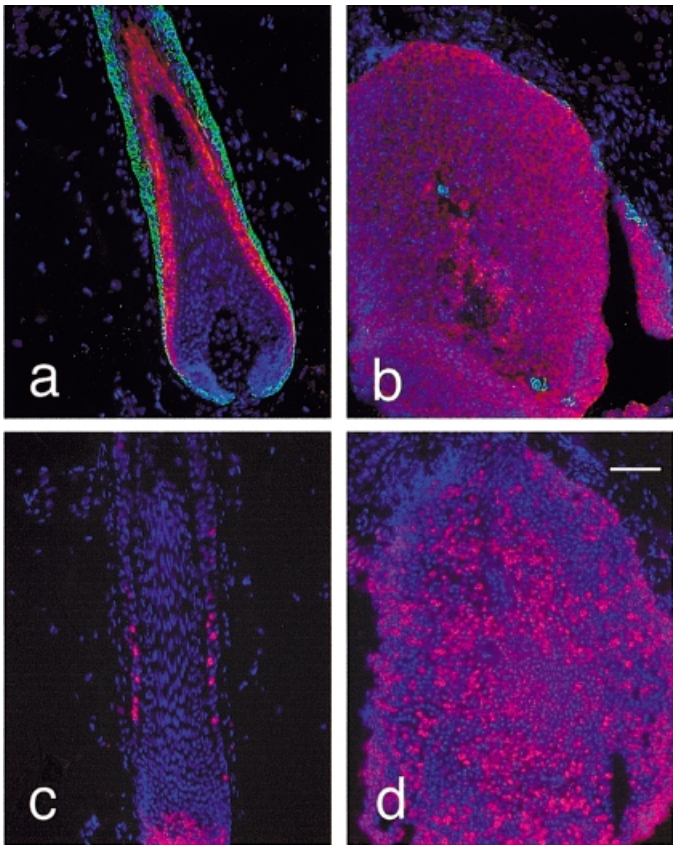

Figure 3. Immunohistochemistry. (*a*) Normal adult follicle stained with AE-15 (Texas red), keratin 14 (FITC-green), and DAPI nuclear stain (blue). (*b*) Patient follicle stained with AE-15 (Texas red), keratin 15 (FITC-green), and DAPI nuclear stain (blue), demonstrating dramatic expansion of the inner root sheath in affected follicles. *Scale bar*: 100 μm. (*c*) Normal adult follicle stained with Ki 67 (Texas red) and DAPI nuclear stain (blue). (*d*) Patient follicle stained with Ki 67 (Texas red) and DAPI nuclear stain (blue), demonstrating marked cell proliferation activity in affected follicles. *Scale bar*: 100 μm.

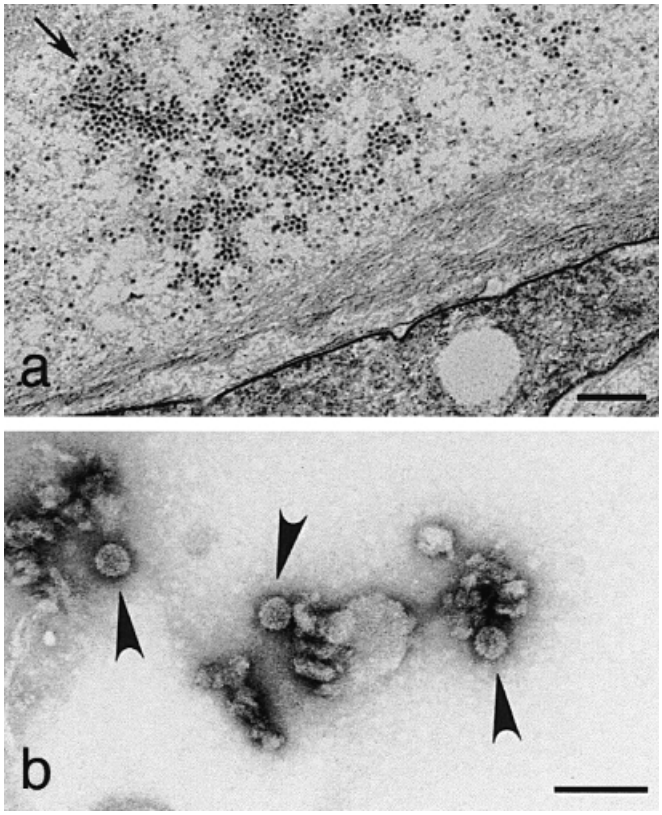

Figure 4. Electron microscopy. (*a*) Transmission electron micrograph of keratinocyte showing viral particles (*arrow*). *Scale bar*: 0.5 μm. (*b*) Electron micrograph of negative stain preparation showing viral particles (*arrows*). *Scale bar*: 100 nm.

subtypes 6/11, 16, 18, 31/33/35/39, 40/42/53/54, 51/52/55/58, and 45/56 did not detect any of these HPV subtypes.

DISCUSSION

Diagnoses considered, based on the clinical presentation alone, initially included alopecia mucinosa, rosacea, ulerythema oophyrogenes, trichostasis spinulosa, and a follicularly based graft *versus* host disease; however, the biopsy findings did not support any of these conditions. Rather, the pathologic changes were due to a folliculocentric infection with a member of the Papovaviridae family. The clinical changes of dramatic alopecia, follicular spinous processes, and indurated papules are, to the best of our knowledge, unique in a human host, and we propose a new name for this entity: ''trichodysplasia spinulosa.''

Morphologically, papillomaviruses and polyomaviruses cannot be differentiated, although polyomaviruses are generally described as being slightly smaller than papillomaviruses (40 *vs* 55 nm). The location of the virally infected cells in the cornifying epithelium in this case would suggest papillomavirus infection. Feline cutaneous papillomavirus infection of aged Persian cats has been reported (Carney *et al*, 1990). These animals presented with reduced hair growth and rough, raised plaques; clinical findings that have some similarities to those in the patient presented here. Polyomavirus must also be considered, however, because of its frequent presence in the urinary tract of kidney and bone marrow transplant recipients (Pappo *et al*, 1996) and because of the small size of the viral particle identified. Furthermore, hair follicle tumors similar to the lesions described here can be experimentally induced with a polyomavirus in laboratory mice (Sundberg *et al*, 1994) and Syrian hamsters (Breuer *et al*, 1993).

To date we have been unable to identify a specific HPV subtype using dot blot hybridization and PCR. This may be because the primers we used are optimized for mucosatropic HPV subtypes that typically infect the genital epithelium. We were also unable to identify any papillomavirus by immunohistochemistry using broadly cross-reactive antipapillomavirus antibodies. It is possible that the tissue sections examined with these techniques were not representative of other sections, such as those visualized by electron microscopy, where virus particles were clearly visible. It is also possible that this is a very antigenically unique papillomavirus that does not cross-react with any of the reagents used. Papillomaviruses are antigenically considered to be very stable, however, and many different mammaliam and avian viruses have been shown to react with the group-specific antibody used here (Lim *et al*, 1990; Jenson *et al*, 1997).

We also tested the tissue for BK virus, which is a polyomavirus, and were unable to detect its presence. Again, as with the papillomaviruses, it is possible that the tissue sections examined for BK virus were not representative of other sections where viral particles were visualized by electron microscopy. Further studies on tissue sections are currently being performed in an attempt to determine the specific virus type, including testing for JC polyomavirus.

Immunosuppression is well known to predispose patients to infection with HPV (Euvrard *et al*, 1997), as well as other viruses. As increasing numbers of individuals receive solid organ transplants and are placed on sustained, aggressive immunsuppressive drug regimens to prevent rejection, it is probable that more cases of trichodysplasia spinulosa will be identified and reported. A number of novel HPV have been identified in the skin of immuno-

suppressed patients (Astori *et al*, 1998). The role these viruses play in cutaneous oncology has yet to be determined.

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